

CLAIMS

1. A method for production and purification of a soluble heterologous fusion protein comprising a cellulose binding module (CBM), from transgenic plants or transgenic plant cells expressing said fusion protein, comprising
- 5 (a) disrupting the transgenic plant material;
- (b) adding an extraction liquid to the plant material, thereby creating a mixture of soluble and insoluble plant material, so as to extract the soluble fusion protein from said disrupted plant material to the liquid phase to obtain a protein extract;
- 10 (c) separating the insoluble plant material, comprising cell-wall material and solids, from said protein extract comprising said fusion protein of interest;
- (d) contacting said protein extract to a polysaccharide matrix which binds to said fusion protein;
- 15 (e) washing the matrix with the bound fusion protein with one or more suitable aqueous solutions; and
- (f) eluting the fusion protein from said polysaccharide matrix by adjusting conditions effecting the release of said fusion protein from the matrix,
- 20 thereby obtaining the soluble heterologous fusion protein substantially purified.
2. The method of claim 1 wherein said transgenic plant or plant cell is selected from the group of dicotyledonous plants and monocotyledonous plants.
- 25 3. The method of claim 1 wherein said plant cell or transgenic plant is selected from the group of plants including tobacco, rape seed, soy bean, alfalfa, lettuce, barley, maize, wheat, oat and rice.
- 30 4. The method of any of claims 1-3, wherein the separation step (c) comprises a method selected from expanded bed adsorption (EBA), precipitation, filtration, centrifugation, or any combination thereof.

5. The method of claim 1 wherein affinity binding to said polysaccharide matrix in step (d) comprises a chromatography step.
- 5 6. The method of claim 1, combining steps (c) and (d) in a process step comprising expanded bed adsorption with a polysaccharide matrix, as a measure for simultaneous separation of cell-wall material and solids from said protein extract and affinity binding of said CBM-fusion protein onto the polysaccharide matrix.
- 10 7. The method of any of claims 1-6, wherein said conditions effecting the elution of said fusion protein from the matrix are non-denaturing conditions that may be neutral or acidic conditions or involve exposure to carbohydrates, or any combination thereof.
- 15 8. The method of any of claims 1-7, wherein said polysaccharide matrix comprises cellulose.
9. The method of claim 8, wherein said cellulose matrix comprises a pharmaceutically compatible cellulose.
- 20 10. The method of claim 9, wherein said cellulose is Avicel™.
11. The method of any of claims 1-10, wherein said transgenic plant or plant cell comprises a nucleic acid sequence encoding for a CBM.
- 25 12. The method of claim 11, wherein said CBM is heat-stable and remains soluble at elevated temperatures.
13. The method of claim 12, wherein said region coding for a CBM is a region of the xylanase10A gene from *Thermotoga maritima*.
- 30 14. The method of claim 13, wherein said region coding for a CBM comprises a sequence depicted as SEQ ID NO: 1, or a sequence encoding the same

amino acid sequence or an amino acid sequence with substantial sequence identity to said sequence.

- 5 15. The method of claim 1, wherein said protein extract is heated to a temperature in the range of 37°C and 100°C, for a period of time in the range of from 1 min to 120 minutes during the process.
- 10 16. The method of claim 16, wherein said heated extract is subjected to the process step comprising expanded bed adsorption with a polysaccharide matrix for the simultaneous separation of solids and affinity binding of said CBM fusion protein from the heated extract.
- 15 17. The method of any of claims 1-16, wherein said heterologous fusion protein comprises a protease.
18. The method of claim 17, wherein said protease is mammalian enterokinase (EK) or an enterokinase active part thereof.
- 20 19. The method of claim 18, wherein said EK comprises a bovine EK catalytic domain (EKc).
20. The method of claim 19, wherein said bovine EKc is encoded by the nucleic acid sequence shown as SEQ ID NO: 2.
- 25 21. The method of claim 1, wherein said fusion protein comprises a CBM and a heterologous polypeptide of interest intercepted by a proteolytic cleavage site.
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